Amendments

In the Specification:

On page 4, on the line following the heading "Brief Description of the Drawings," please add the following paragraph:

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Please replace the paragraph beginning on page 12, line 20 with the following paragraph:

In Figures 5A and 5B, solutions obtained with co-crystals of elastase inhibitors are compared with data obtained by the methods herein described. In Figure 5A, the solutions for six co-crystallized inhibitors are shown, with the inhibitor molecules overlaid on each other (non-space-filling representation, with the elastase segment represented by a space-filling illustration). These inhibitors are trifluoroacetyl-l-lysyl-l-prolyl-p-isopropylanilide (Mattos, C. *et al.*, *Nat. Struct. Biol.* 1:55-58 (1994); crystal solution: PDB ID: 1ELA ~~Mattos et al., as submitted April 30, 1994~~), trifluoroacetyl-l-lysyl-l-leucyl-p-isopropylanilide (Mattos, C. *et al.*, *Nat. Struct. Biol.* 1:55-58 (1994); crystal solution: PDB ID: 1ELB ~~Mattos et al., as submitted June 22, 1994~~), trifluoroacetyl-l-phenylalanyl-p-isopropylanilide (Mattos, C. *et al.*, *Nat. Struct. Biol.* 1:55-58 (1994); crystal solution: PDB ID: 1ELC ~~Mattos et al., as submitted April 30, 1994~~), trifluoroacetyl-l-phenylalanyl-l-alanyl-p~~-trifluoromethylaninide~~ p-trifluoromethylanilide (Mattos, C. *et al.*, *Biochemistry* 34:3193-203 (1995); crystal solution: PDB ID: 1ELD ~~Mattos et al., as submitted February 14, 1995~~),

trifluoroacetyl-1-valyl-1-alanyl-p-trifluoromethylanilide (Mattos, C. *et al.,* *Biochemistry 34*:3193-203 (1995); crystal solution: PDB ID: 1ELE ~~Mattos et al., as submitted February 14, 1995~~) and n-(tert-butoxycarbonyl-alanyl-alanyl)-o-(p-nitrobenzoyl) hydroxylamine (Ding, X. *et al., Biochemistry 34*:7749-56 (1995); crystal solution: PDB ID: 1ELF ~~Ding et al., as submitted July 10, 1995~~). In Figure 5B, the solutions for approximately 10 ORFs, which are in their respective high affinity protein binding states are overlaid. Both methods identify a region which favors the binding of aromatic moieties. The simulation process achieves approximately 90% 3D geometric identity with the crystallography results.

Please replace the paragraph beginning on page 17, line 2 with the following paragraph:

Aspects of the simulations used in the invention can be illustrated with calculations used to determine the strength of water binding to a synthetic polynucleotide (Guarnieri, F., and Mezei, M., *J. Am. Chem. Soc. 118*:8493-8494 (1996)).[[¹]] This illustration can be described as follows:

Please replace the paragraph beginning on page 17, line 10 with the following paragraph:

Grand canonical ensemble simulations are generally performed by placing a molecule in a periodic simulation cell, setting a parameter B, which is representative of free energy, in such a way as to achieve an experimentally determined density, sampling potential hydration positions around the molecule by inserting and deleting water molecules from the simulation cell using a technique such as cavity-bias (Mezei, M., *Mol. Phys. 61*:565-582 (1994); Resat, H., and Mezei, M., *J. Am. Chem. Soc. 116*:7451-7452

(1994)),[[2,3]] and accepting or rejecting the attempt based on a Metropolis Monte Carlo (Metropolis, N. *et al., J. Chem. Phys. 21*:1087-1092 (1953))[[4]] criteria using a grand canonical ensemble probability function (Tolman, R., in *The Principles of Statistical Mechanics*, Dover Press, New York (1971)).[[5]] The parameter B is related to the excess chemical potential μ' as follows: $B = \mu'/kT + ln<N>$, where k is Boltzmann's constant, T is the absolute temperature, and $<N>$ is the mean number of molecules of the ORF, which here is H_2O. In the method of *simulated annealing of chemical potential*, the simulation is started with a large initial B-value so that a higher percentage of water insertion attempts are accepted. This causes the simulation cell to be flooded with water molecules. After this grand canonical ensemble simulation at high excess chemical potential is equilibrated, subsequent simulations are carried out at successively lower B-values. This successive lowering of the B-values causes a gradual removal of the bulk water molecules from the simulation cell. As the chemical potential is further "annealed", a point is reached at which water molecules do not readily leave the cell, thereby identifying those water molecules that are strongly influenced by the DNA, the so-called "bound water molecules". As the excess chemical potential is again lowered, ultimately some of these bound waters start to leave the cell. Since chemical potential is a free energy, this *simulated annealing of chemical potential* yields a numerical estimate of the differential free energy of binding of the different bound water molecules. It must be emphasized that our utilization of the term "annealing" applies strictly to the value of the chemical potential and that the temperature is kept constant at, for example, 298 K in all the simulations. For all simulations the DNA was held fixed, water molecules were added and deleted throughout all parts of the cell,

extensive canonical Monte Carlo was performed between accepted grand canonical Monte Carlo steps, and periodic boundary conditions were used.

Please replace the paragraph beginning on page 18, line 6 with the following paragraph:

As an illustration of the method, a *simulated annealing of chemical potential* on a d(CGCGAATTCGCG)$_2$ was performed, starting with $B = 1.0$ down to -26 in 37 increments performing 2,000,000 cavity-biased grand canonical ensemble Monte Carlo steps at each B-value. The final configuration of the simulation with $B = -6$, has 1120 bound water molecules. The final configuration of the simulation with $B = -8$, has 533 bound water molecules. The final configuration of the simulation with $B = -9$, has 390 bound water molecules. The final configuration of the simulation with $B = -11$, has 215 bound water molecules. The most salient feature of this progression is the differential hydration of the major and minor groove of the DNA. The $B = -6$ simulation shows the DNA essentially uniformly solvated. The $B = -8$ simulation clearly shows that upon lowering of the chemical potential by 2 B-units, a majority of the nonbulk extracted waters come from the major groove, while the minor groove remains almost unaffected. Annealing the chemical potential further ($B = -9$) still leaves the minor groove well hydrated while the major groove is almost stripped. Lowering B even further ($B = -11$) results in the removal of almost all water molecules from both the major and minor groove. Quantitation of the hydration of the DNA as a function of chemical potential was computed by proximity analysis (Mehrotra, P.K., and Beveridge, D.L., *J. Am. Chem. Soc. 102*:4287 (1980); Mezei, M., *Mol. Simul. 1*:327-332 (1988) (describing the effects of different partial charges on proximity analysis))[[6,7]] with the results shown in Table 1:

| | first hydration shell | | | | first and second hydration shell | | | |
| | minor groove | | major groove | | minor groove | | major groove | |
B	no. of waters	density	no. of waters	density	no. of waters	density	no. of waters	density
-6	7.27	0.013	13.23	0.012	21.3	0.021	41.7	0.011
-8	5.4	0.010	5.06	0.004	14.6	0.015	11.8	0.003
-9	4.08	0.007	4.36	0.004	11.5	0.011	9.7	0.003
-11	1.04	0.002	2.11	0.002	3.9	0.004	4.2	0.001

For $B = -6$, the first hydration shell (defined by the position of the first minimum of the radial distribution function) of the major and minor groove has a comparable density (0.012 and 0.013, respectively), while the second hydration shell of the minor groove has twice the density of the major groove. For $B = -8$ the hydration difference becomes quite pronounced with the minor groove first and second shell hydration density being 2.5 fold and 5 fold higher than the major groove, respectively. For $B = -11$ the major and minor groove hydration density again becomes equal because at this value of the excess chemical potential both grooves are essentially stripped bare.

Please replace the paragraph beginning on page 19, line 6 with the following paragraph:

Illustrating the differential hydration propensities of the major and minor grooves of DNA is computationally undemanding (3 days of CPU time to run one annealing schedule and 3 days of CPU time to run one proximity analysis (Mezei, M., and Beveridge, D.L., in *Methods in Enzymology*, Packer, ed., Academic Press, New York, pp. 21-47 (1986) (describing the effects of volume element calculations—which can be CPU intensive—on

proximity analysis))[[[7]]] on an SGI Power Challenge) using *simulated annealing of chemical potential* because only a coarse "cooling" schedule of the chemical potential is required. Since the chemical potential is a free energy, a very fine cooling schedule may be used to estimate quantitatively the hydration free energy difference of two different functional groups or even two different atoms of the DNA. Two atoms that desolvate at the same B-value have similar solvation free energy, or alternatively, require a finer cooling schedule to resolve the differences. It should be noted that the model system used here consisted of ionic DNA with 22 negative charges and no sodium counterions. The findings presented herein about the preferential hydration of the minor groove corresponds very well to results from X-ray crystallographic and NMR studies. Possible reasons for the stronger binding of water molecules in the minor groove may include the following: the high density of the charged rows of phosphate groups, steric constraints, and specific water—water, water—DNA interactions.

Please replace the paragraph beginning on page 21, line 6 with the following paragraph:

References

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references. ~~The cited documents incorporated by reference into this disclosure include:~~

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[1] Guarnieri and Mezei, *J. Am. Chem. Soc.* 118: 8493-8494, 1996.

[2] Mezei, *Mol. Phys.* 61: 565-582, 1994.

[3] Resat and Mezei, *J. Am. Chem. Soc.* 116: 7451-7452, 1994.

[4] Metropolis et al., *J. Chem. Phys.* 21: 1087-1092, 1953.

[5] Tolman, R. In *The Principles of Statistical Mechanics*, Dover Press, New York, 1971.

[6] Mehrotra and Beveridge, *J. Am. Chem. Soc.* 102: 4287, 1980.

[7] The effects of different partial charges on proximity analysis are described in: Mezei, *Mol. Simul.* 1: 327-332, 1988.

[8] Calculations of volume elements can be CPU intensive. The effects of volume element calculations on proximity analysis are described in Mezei and Beveridge In *Methods in Enzymology*, Packer, Ed., Academic Press, New York, 1986, pp. 21-47.